THE CHEMISTRY OF THE *ARISTOLOCHIA* SPECIES. PART V. A COMPARATIVE STUDY OF ACIDIC AND BASIC CONSTITUENTS OF *A. RETICULATA* LINN., *A. SERPENTARIA* LINN., *A. LONGA* LINN. AND *A. INDICA* LINN.

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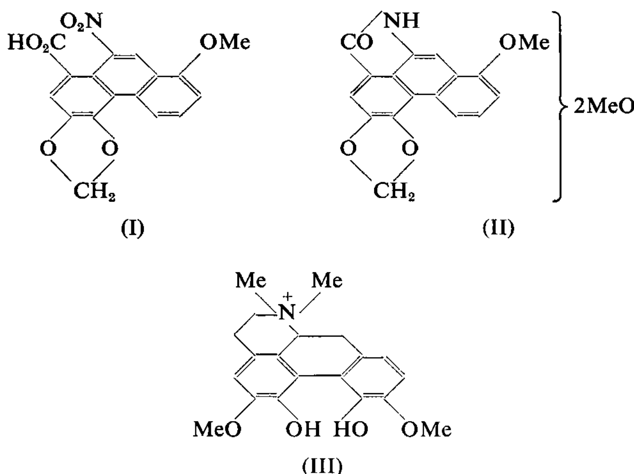
Aristolochic acid (I) was present in single samples of *A. longa* and *A. indica*, in two samples of *A. serpentaria*, and four samples of *A. reticulata*. Aristo-red (II) was present only in the samples of *A. reticulata* and *A. serpentaria*. Contrary to numerous reports of alkaloids in *Aristolochia* species, the current investigation has revealed the presence of only negligible amounts in the samples of *A. indica*, *A. longa*, *A. reticulata* and *A. serpentaria* examined. A flavone isolated from *A. reticulata* has been identified as isorhamnetin.

In Part III[1] acidic material from the roots and rhizomes of *A. reticulata* was shown to contain both aristolochic acid (I)[2,3] and aristo-red (II) whereas only the former was found in *A. indica*. Aristolochic acid and aristo-red have now also been separated by fractional crystallisation of



the acid isolated from *A. serpentaria*, and identified by comparison with authentic samples. The controversial reports of Pohl[4], who described the presence of aristolochin (≡ aristolochic acid[5]), and of Hesse[6], who was unable to identify aristinic acid (≡ aristolochic acid) in *A. longa*, have been resolved by the isolation of aristolochic acid (I) in good yield from this source. This, and other data summarised in Table I, indicates that aristolochic acid is characteristic of all *Aristolochias* so far examined. Aristo-red (II) is not present in *A. longa* and, like aristolactone[7], appears to be a characteristic constituent only of the North American *Aristolochias*.

TABLE I

ACIDS AND LACTONE CONSTITUENTS OF *Aristolochia* SPECIES

Source	Aristolochic acid (per cent)	Secondary acid constituents	Aristolactone (per cent)	Ref.
A. argentina, Griseb.	*	–	–	6
A. bracteata, Retz.	0·01	–	–	9
A. clematitis, Linn.	*	aristolochic acid–II	–	2; 3; 10
A. debilis, Sieb. et Zucc.	*	–	–	11
A. indica, Linn.	0·013	–	–	12
	0·070	0	0	1; 7
A. kaempferi, Willd.	*	–	–	13
A. longa, Linn.	0·20	0	0	7; present work
A. reticulata, Linn.	0·022	aristo-red	0·158	1; 7
A. serpentaria, Linn.	0·046	aristo-red	0·091	7; present work
	0·92	–	–	31
A. sipho, l'Hérit.	0·30	–	–	5
A. maxima, Jacq.	*	–	–	31
A. pandurata, Jacq.	*	–	–	31

* Present in unstated amount
– No specific search recorded
0 Substance absent

Cavallito and Bailey[8] isolated a crystalline substance, $C_{46}H_{11}O_7N$, from *Asarum canadense var. reflexum* (fam. Aristolochiaceae) which they termed substance B, the properties of which strongly suggest identity with aristolochic acid (see Table II).

TABLE II

PROPERTIES OF SUBSTANCE B AND ARISTOLOCHIC ACID

	Substance B	Aristolochic acid
Appearance	Yellow needles	Yellow needles
m.p.	Darkens between 230–260° without melting	Darkens around 240–250° then melts at 275° (decomp.)
Analyses	Found: C, 58·2 H, 3·5 N, 4·55 per cent	Requires C, 59·8 H, 3·2 N, 4·1 per cent
Ultra-violet absorption spectrum	λmax. (mμ) / ε (based on $C_{17}H_{11}O_7N$) 250 / 29,325 318 / 12,820 390 / 6,615	λmax (mμ) / ε 223 / 30,000 250 / 29,400 318 / 13,100 390 / 7,300

Alkaloids have been reported from time to time to be present in various *Aristolochia* species, since Feneulle[14] and Chevallier[15] described the isolation of a bitter yellow substance from *A. serpentaria.* Neither author claimed that this substance was basic, though later, Ferguson[16] indicated that it was probably identical with the bitter yellow crystalline base, aristolochine, which he obtained from *A. reticulata.* Winkler[17], on the other hand, claimed that the yellow crystalline acid from *A. clematitis*, which was undoubtedly aristolochic acid, was also identical with the bitter obtained by Feneulle and Chevallier. Hesse[6] reported the isolation from *A. argentina* and *A. indica* of an amorphous base, aristolochine, different from Ferguson's aristolochine, and also stated that alkaloids were not present in *A. longa.* Controversial reports are

also on record concerning the presence[18] or absence[19] of alkaloids in *A. cymbifera*.

A base was also reported by Dymok and Warden[20] in *A. indica* and later isolated in crystalline form, $C_{17}H_{19}O_3N$, from the same source by Krishnaswamy, Manjunath and Rao[12,21] in a yield of 0·05 per cent. The alkaloid which was also named aristolochine gave a crystalline hydrochloride, picrate (m.p. 222° decomp.) and picrolonate, showed the presence of one methoxyl group, an *N*-dimethyl group, and exhibited weakly acidic properties (soluble in sodium hydroxide, but insoluble in sodium carbonate), but gave no ferric chloride reaction. No detailed structural analysis has been reported. More recently, Tomita and Kura[13] have described the isolation of an aporphine type base, magnoflorine (III)[22] from *A. debilis* Sieb. et Zucc. and *A. kaempferi* Willd.

Plants of the genus *Aristolochia* are reported to have been held in high esteem on account, among other things, of their value in childbirth[23], and the common name Birthwort for certain species is obviously derived from these traditional uses. Some credence to the use of *Aristolochia* for this purpose is given by the work of Shaw[24], who reported that *A. elegans* contained an alkaloid which caused contraction of the uterus. We have accordingly examined a number of *Aristolochias* for alkaloids of potential value as oxytocics.

Single samples only of *A. longa* and *A. indica*, but two samples of *A. serpentaria* (contaminated with *Hydrastic canadensis*, from which it was separated as described below), and four samples of *A. reticulata*, received over a period of six years, were available to use. We are indebted to Dr. F. Fish and Mr. P. F. Nelson of this College for confirming the authenticity of the species designation of the samples. Index Kewensis lists four species of *Aristolochia longa*, and we wish to thank Dr. C. R. Metcalfe of the Royal Botanic Gardens, Kew, for his further confirmation of the authenticity of our sample as *A. longa* Linn.

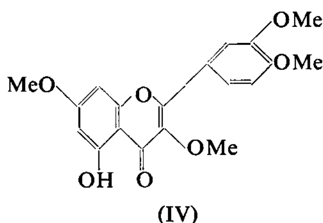
The ethanolic extract from *A. reticulata* gave only negligible amounts of a dark chloroform-soluble base, but precipitation of the residual aqueous liquor gave a crude reineckate, which was purified by chromatography from acetone on alumina. Decomposition of the pure reineckate[25], $C_{17}H_{20}O_3N[Cr(SCN)_4(NH_3)_2]\cdot 3H_2O$, yielded a hygroscopic partly crystalline base chloride, which gave a strongly positive reaction with Mayers reagent, but analysed only indifferently to the formula $C_{17}H_{20}O_3NCl$. A picrate, m.p. 178–179·5°, insufficient for analysis, was also obtained. Thus, although the molecular formula corresponds to that of the base reported in *A. indica*[12,21] inconsistency of the picrate melting points suggests that they are not identical.

A. indica yielded only a very small quantity (0·0007 per cent) of a yellow ether-soluble crystalline base, m.p. 339–342° (decomp.). The substance which was only weakly basic, fluoresced under ultra-violet light, and analysed as $C_{25}H_{23}O_{10}N$, again indicating non-identity with the alkaloid previously described[12,21]. Water-soluble alkaloids were absent. It is perhaps significant that the sample of root examined contained a higher percentage of aristolochic acid than reported by Krishnaswamy

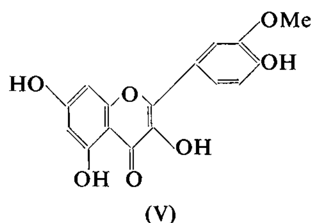
and others[12] (see Table I), and since the acid ($C_{17}H_{11}O_7N$) would appear to be biogenetically related to the base they described ($C_{17}H_{19}O_3N$), our failure to find this alkaloid may possibly be explained by the time of year at which the plant material was collected. This aspect of the problem is being examined further.

Examination of the sample of *A. serpentaria* before extraction revealed that it was contaminated with *Hydrastis canadensis* root, and despite hand picking of this sample to remove contaminants, it yielded on extraction small quantities of hydrastine, together with a second base. Hydrastine was identified by analysis, melting point and ultra-violet absorption spectrum, but yielded a picrate, the melting point of which, 149°, was not in agreement with the reported values of 184°[26] and 190°[27]. Preparation of authentic hydrastine picrate from a sample of Liquid Extract of Hydrastis B.P.C. 1949 showed the melting point to be 149°. The second base, m.p. 178–179° was formulated by analysis as $C_{18}H_{15}O_{10}N$, and is not therefore identifiable with berberine or canadine the other known constituents of *Hydrastis canadensis*[28], or indeed any other known base. It was only weakly basic and showed an ultra-violet absorption maxima at 281·5 mμ (ε 12,030) and 353 mμ (ε 13,365) of the berberine type[29]. A second sample of *A. serpentaria*, which was similarly contaminated with *Hydrastis canadensis* yielded both hydrastine and berberine (in approximately equal amounts as usually found), but failed to yield the alkaloid $C_{18}H_{15}O_{10}N$. It is not clear, therefore, whether this base is present in *A. serpentaria* or derives from some further contaminant. In agreement with the findings of Hesse[6], no alkaloids were found in *A. longa.*

Further fractionation of the crude ethanol-soluble material from *A. reticulata*, isolated as described in Part III[1], yielded a small quantity of a yellow crystalline amphoteric substance, $C_{16}H_{12}O_7$, m.p. 318–322°, raised to 324° on repeated sublimation. The product was soluble in concentrated sulphuric acid to give a deep yellow solution, gave a greenish-brown colour with ferric chloride, and showed maxima in the ultra-violet at 255 mμ (ε 21,150), 307 mμ (ε 7,950) and 371 mμ (ε 22,100), all characteristic of a hydroxyflavone. Zeisel determination showed the



(IV)



(V)

presence of one methoxyl group, and it was identified as a tetrahydroxy-methoxyflavone by conversion to the corresponding tetra-acetate, m.p. 214–215°, with acetic anhydride-pyridine. Diazomethane, on the other hand, provided evidence of a non-reactive hydroxyl, typical of 5-hydroxy-flavones[30] giving 5-hydroxy-3,3′,4′,7-tetramethoxyflavone (quercetin-3,3′,4′,7-tetra-methyl ether (IV)) as shown by melting point, ferric

chloride reaction, ultra-violet absorption spectrum and elementary analysis.

Identification of the parent monomethoxytetrahydroxyflavone, however, was not immediately possible, since although all five possible monomethoxyquercetins are known, the observed constants of our own product and its tetra-acetate did not conform with those of the derivatives for which corresponding data is available (Table III).

TABLE III

MONOMETHOXYQUERCETINS AND THEIR TETRA-ACETATES

Substance	m.p. (° C)	Ref.	λmax	log ε	Ref.	Tetra-acetate m.p. (° C)	Ref.
Quercetin-7-methyl ether (Rhamnetin) ..	294–296 292–293 >300 290–294	39 40 34 42	256 371	4·40 4·41	32 32	186–188 186–187 190–192 183–185	39 40 34 42
Quercetin-3′methyl ether (Isorhamnetin) ..	296 305 307 295	41 37 38 35	255 365–380 (flat)	— —	36 36	198–199 205–207 205 198–200	41 37 38 35
Quercetin-4′-methyl ether	240 259–260	43 30				202 203–204	43 30
Quercetin-5-methyl ether			254 369	4·30 4·25	32 32		
Quercetin-3-methyl ether	272–273	33	258 360	4·31 4·31	32 32		
Quercetin-*x*-methyl ether .. .. .. (present work)	318–322 (block)		255 370–372	4·32 4·34		214–215 (block)	

The considerable variation of recorded melting points is due to the fact that they are accompanied by decomposition, which makes them unreliable for characterisation purposes. Nevertheless, it would appear improbable that our own product is either the 3- or the 4′-methyl ether, whilst the 5-methyl ether is also excluded since diazomethane would yield pentamethoxy- and not the tetramethoxy-quercetin. The former conclusion is substantiated by the instability of the parent compound in ethanolic sodium ethoxide, which can be followed spectroscopically and is characteristic of flavones with unsubstituted hydroxyls in both the 3- and 4′-positions[32]. The ultra-violet spectrum in ethanolic sodium ethoxide (Fig. 1) differs from that recorded for rhamnetin (inflection at 238 mμ, log ϵ 4·35; λmax 294, log ϵ 4·11; λmax 358, log ϵ 3·98)[32]. There are no published spectra for isorhamnetin under the same conditions. The spectrum in ethanol is unaffected by boric acid-sodium acetate. Spectral shifts with the latter reagent are typical of vicinal dihydroxy compounds[46], and failure to elicit a shift is indicative of a methoxyl in the 3′-position and hence of isorhamnetin (V). Identity with isorhamnetin also seems to present the most reasonable conclusion on grounds of melting point (Table III) and ultra-violet absorption spectra, for although the spectra of rhamnetin and isorhamnetin are very similar, the former has a very broad minimum in the 300 mμ region[32], whereas the latter has a sharp minimum near 290 mμ[44].

An authentic sample of isorhamnetin could not be isolated by the reported method[45] from powdered red squill. Samples of synthetic isorhamnetin and its tetra-acetate were obtained, however, through the kindness of Professor G. Tappi[36]. In appearance, they were identical with our own products. Microblock melting points of 318–320° and 210–211° respectively were also in excellent agreement, whilst the ultra-violet absorption spectra of the synthetic isorhamnetin and our own flavone were superposable (Fig. 1), thus confirming identity.

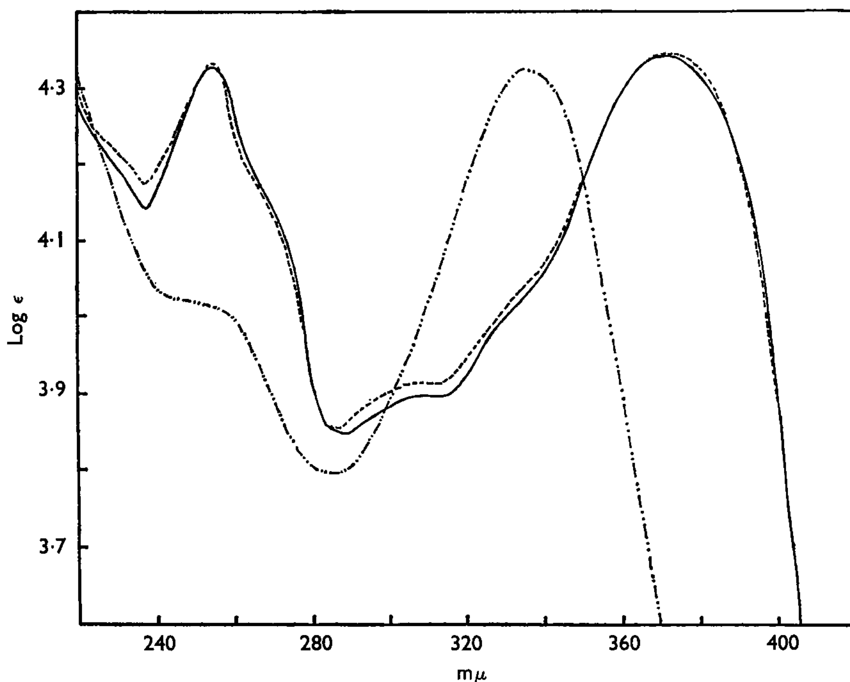


FIG. 1. Ultra-violet absorption spectra. Isorhamnetin - - - -. Flavone from *A. reticulata* ——. Flavone from *A. reticulata* in ethanolic sodium ethoxide – · · –.

EXPERIMENTAL

Melting points are uncorrected. Ultra-violet absorption spectra were determined in absolute ethanol on a Hilger Uvispek photoelectric spectrophotometer. R_F values were determined on Whatman No. 1 paper with 4:1 ethanol:5 per cent formic acid as solvent. We thank Mr. W. McCorkindale and Dr. A. C. Syme for microanalyses.

Extraction of A. longa

The dried root (3·01 kg.; No. 60 powder), previously defatted with light petroleum (b.p. 40–60°), was extracted with ethanol by cold percolation to give a dark orange extract (10 l.). During concentration, the yellow crystalline acid which separated out (7·12 g.) was repeatedly

filtered off before an almost black thick oil was obtained. The residue was acidified with dilute hydrochloric acid and the crude acids extracted with ether (treatment of the acid extract is reported below). Extraction of the ethereal solution with 2 per cent aqueous potassium hydrogen carbonate followed by acidification of the aqueous layer with dilute hydrochloric acid gave the crude acids. Fractional crystallisation of the bulked acid portions from glacial acetic acid gave eight fractions as yellow microcrystals (total weight 6·01 g.), each with m.p. 282–285° (decomp., block), R_F 0·90–0·94, λmax 250 (ϵ 30,600), 317 (ϵ 11,500), 390 mμ (ϵ 5,700), identical with aristolochic acid. Reduction with zinc and glacial acetic acid gave 9-amino-1-methoxy-5,6-methylenedioxy-8-phenanthroic lactam, m.p. 317° (block).

Treatment of acid extract. The solution was basified (dilute sodium hydroxide) and extracted with ether which on evaporation gave only a trace of a brown non-alkaloidal oil. The aqueous layer was acidified to congo red (dilute sulphuric acid) and treated with a saturated aqueous solution of ammonium reineckate. The resultant crude precipitate (4·078 g.) was completely insoluble in dry acetone.

Extraction of A. serpentaria

(*a*) The first sample of dried root and rhizome (4·34 kg.), from which appreciable quantities of *Hydrastis canadensis* root and rhizome and other adulterants had been removed, was reduced to a No. 60 powder, defatted with light petroleum (b.p. 40–60°) and percolated in the cold with ethanol until the percolate was pale brown (7 days). The thick black oil obtained on concentration was left at 0° for 4 days during which time β-sitosteryl-β-D-glucoside (1·88 g.) separated as a brown crystalline solid, m.p. 295–296° (after repeated recrystallisation from ethanol); acetate m.p. 166°. [Kind and Celentano[47] give m.p. of 295–297°, 167·5–168·5° respectively for β-sitosteryl-β-D-glucoside and its tetra-acetate.] The oily filtrate was dissolved in ether and the solution extracted with dilute hydrochloric acid (treatment of this acid extract is reported below). The crude acid fraction (3·64 g.), obtained from the ether solution by the method used for *A. longa*, was recrystallised from glacial acetic acid and gave aristolochic acid (2·00 g.), m.p. 283° (decomp.; block), R_F 0·915, identified further by conversion to 1-methoxy-5,6-methylenedioxy-9-nitrophenanthrene, orange needles, m.p. 212° (block). Concentration of the glacial acetic acid mother liquors gave, on repeated fractional recrystallisations from ethanol, red needles of aristo-red (35 mg.), m.p. 286·5–287·5° (block), R_F 0·78 (fluorescent spot in ultra-violet light). The ultra-violet absorption spectrum agreed with that reported in Part III[1].

Treatment of acid extract. The acidic solution was basified (dilute ammonium hydroxide) and extracted with ether, which on evaporation gave a dark-red partially crystalline oil (500 mg.). The benzene-soluble portion was chromatographed on alumina (5 in. × 0·5 in.) from benzene to give two fractions. The benzene-insoluble portion was non-alkaloidal.

Fraction 1. This came through as a compact yellow band which on evaporation gave pale yellow prism crystals (74 mg.), m.p. 178–179°

(decomp.; tube) (from ether or benzene). λmax 281·5 (ε 12,030), 353 mμ (ε 13,365). (Found: C, 53·6; H, 3·75; N, 3·6. $C_{18}H_{15}O_{10}N$ requires: C, 53·4; H, 3·7; N, 3·5 per cent.

Fraction 2. Removal of benzene gave pale yellow prism crystals of hydrastine (62 mg.), m.p. 132° (tube) (from methanol). λmax 297 mμ [*E* (1 per cent, 1 cm.) 196]. (Found: C, 65·6; H, 5·6; N, 3·7. Calculated for $C_{21}H_{21}O_6N$: C, 65·8; H, 5·5; N, 3·7 per cent.) [El Ridi, Khalifa and Mamoon[48] gave λmax 297 mμ [*E* (1 per cent, 1 cm.) 200, m.p. 132°]. The picrate had m.p. 148–149° (tube) (from ethanol). (Found: C, 53·2; H, 4·25. Calculated for $C_{21}H_{21}O_6N{\cdot}C_6H_2(NO_2)_3OH$: C, 52·95; H, 3·95 per cent.

(*b*) The second sample of defatted root and rhizome (4·20 kg., No. 60 powder), on concentration of the ethanolic extract, gave a thick black oil from which aristolochic acid, aristo-red and the acid extract were obtained as before.

Treatment of acid extract. The solution was basified (dilute sodium hydroxide) and extracted into ether which was, in turn, shaken out with sulphuric acid (2·5 per cent). On standing, the aqueous layer deposited orange crystals of berberine sulphate (1·037 g.), m.p. 288–290° (decomp.; block) (from alcohol-ether), λmax 267 [*E* (1 per cent, 1 cm.) 648], 351 mμ [*E* (1 per cent, 1 cm.) 609] (in 88 per cent ethanol). El Ridi, Khalifa and Mamoon[48] gave λmax 270 [*E* (1 per cent, 1 cm.) 610], 350 mμ [*E* (1 per cent, 1 cm.) 600] for berberine hydrochloride. (Found: C, 55·0; H, 4·2; N, 3·3; S, 7·2. Calculated for $C_{20}H_{17}O_4N{\cdot}H_2SO_4$: C, 55·4; H, 4·4; N, 3·2; S, 7·4 per cent.) The ether layer gave yellow prism crystals on removal of the solvent and chromatography from benzene on alumina (6 in. × 0·5 in.) yielded only hydrastine (1·097 g.), m.p. 132° (tube) (from methanol), 145° (tube) (from aqueous methanol). Both melting points have been reported[27] for hydrastine. The picrate melted at 148–149°.

The acid extracts from both samples of *A. serpentaria*, which had been basified and extracted with ether to remove basic material, were re-acidified to congo red (dilute sulphuric acid). Addition of a saturated aqueous solution of ammonium reineckate gave an amorphous dark brown solid (5·133 g.), which was only slightly soluble in dry acetone. Chromatography from dry acetone on alumina (38 g., 6·5 in. × 0·75 in.) gave a negligible quantity of pure reineckate.

Hydrastine picrate. A sample of hydrastine (0·82 g.), m.p. 132°, was obtained from Liquid Extract of Hydrastis B.P.C. 1949 (50 ml.) using the official assay method. The picrate was prepared by dissolving the base (0·1 g.) in hot methanol (10 ml.) and adding a saturated solution of picric acid in ethanol (5 ml.). It had m.p. 149° (decomp., tube) (from ethanol).

Extraction of A. reticulata

Treatment of acid extract. This was obtained by the method reported in Part III[1]. The acidic solution after a few days was basified (dilute ammonium hydroxide) and extracted with ether. After extraction of the latter with dilute hydrochloric acid to remove bases (treatment of this

fraction is reported below) the ether was evaporated to give yellow microcrystals of isorhamnetin (0·54 g.), m.p. 318–322° (decomp.; block) (from dioxan), raised to 324° on repeated sublimation at 300°/0·5 mm. (Found: C, 60·8; H, 3·7; O, 35·9; OCH_3, 11·5. Calculated for $C_{15}H_9O_6{\cdot}OCH_3$: C, 60·8; H, 3·8; O, 35·45; OCH_3, 9·8 per cent.) λmax 255 (ε 21,150), 307 (ε 7,950), 370–372 mμ (ε 22,100). The ultra-violet absorption spectrum in ethanolic sodium ethoxide was carried out by the method of Jurd and Horowitz[32] allowing 1 hr. for reaction, λmax 335 (ε 21,200), 250–252 mμ (ε 10,480 flat). The spectrum in presence of boric acid-sodium acetate was recorded using the method of Jurd[46]. [A sample of isorhamnetin obtained from G. Tappi[36] had m.p. 318–320° (decomp., block), λmax 255 (ε 21,250), 307 (ε 8,150), 370–372 mμ (ε 22,120.] The flavone was also obtained by leaving the original acid extract at room temperature for several days when a green oily precipitate separated. Sublimation at 300°/0·5 mm. gave isorhamnetin (35 mg.).

Treatment of solution containing basic material. Successive extractions with ether then chloroform gave only traces of a dark-brown oil which gave slight positive tests with alkaloidal reagents.

The original acid extract, which had been basified and extracted with ether, was re-acidified to congo red (dilute sulphuric acid) and to it was added in excess a saturated aqueous solution of ammonium reineckate. The dark-brown crude base reineckate (31·2 g.) was dissolved in dry acetone and filtered from a large quantity of non-alkaloidal material. The deep red acetone solution was chromatographed from dry acetone on alumina (20 in. × 1·3 in.) and the single red band evaporated (water-bath, $< 50°$) to give a pink crystalline reineckate, m.p. 200°, (decomp., tube, insert at 195°) (from aqueous acetone). (Found: C, 37·8; H, 4·8; N, 14·8; OCH_3, 4·1, 4·0. $C_{16}H_{17}O_2N(OCH_3)$ $[Cr(SCN)_4(NH_3)_2]{\cdot}3H_2O$ requires C, 38·3; H, 4·9; N, 14·9; OCH_3, 4·7 per cent.)

Decomposition of base reineckate. The reineckate (0·79 g.) was dissolved in dry acetone (20 ml.) and excess solution of silver sulphate added (0·599 per cent w/v, 35·0 ml.) followed by an equivalent volume of a solution of barium chloride (1·062 per cent w/v $BaCl_2{\cdot}2H_2O$; 15·50 ml.) when precipitation of silver reineckate had ceased. The combined precipitates of silver reineckate and barium sulphate were filtered off and washed thoroughly with distilled water; the combined filtrate and washings were evaporated to dryness (water-pump). This gave a very hygroscopic partially crystalline solid of doubtful purity (0·216 g.) from which inorganic material could not be completely removed. After repeated solution in water, it had $[\alpha]_D^{18} + 50{\cdot}83$, λmax 228 [*E* (1 per cent, 1 cm.) 367], 286 mμ [*E* (1 per cent, 1 cm.) 122]. (Found: C, 61·0; H, 9·2; N, 5·6. The expected base chloride $C_{17}H_{20}O_3NCl$ would require: C, 63·4; H, 6·3; N, 4·4 per cent.)

Base picrate. The base (50 mg.) was dissolved in water (2 ml.) and to this solution was added an aqueous solution of picric acid (0·66 per cent w/v, 4 ml.). Recrystallisation of the bulky product from ethanol was accompanied by decomposition and gave crystals (4 mg.), m.p. 178–179·5° (decomp., block).

Quercetin—3,3′4′,7-tetramethyl ether. Isorhamnetin (40 mg.) was suspended in dry ether (12 ml.) and an excess of diazomethane in dry ether added but no reaction occurred until a drop of water was added as catalyst[51]. After 3 hr., the excess diazomethane and solvent was removed giving long pale-yellow needles (19 mg.), of quercetin-3,3′4′,7-tetramethyl ether, m.p. 159–160° (tube) (from ethanol). (Found: C, 63·5; H, 5·4. Calculated for $C_{19}H_{18}O_7$: C, 63·7; H, 5·1 per cent.) λmax 254 (log ϵ 4·33), 269 (log ϵ 4·26), 353 mμ (log ϵ 4,305). [Gomm and Nierenstein[49] gave m.p. 159–160°. Briggs and Locker[50] gave λmax 254 (log ϵ 4·37), 269 (log ϵ 4·29), 352 mμ (log ϵ 4·34).]

Isorhamnetin—3,4′,5,7-tetra acetate. Isorhamnetin (40 mg.) was refluxed for 30 min. with acetic anhydride (2 ml.) and pyridine (2 ml.). To the cooled mixture, water was added dropwise to give white needles (72 mg.), which fluoresced brilliant green in ultra-violet light and had m.p. 214–215° (block) (from ethanol), λmax 239 (ϵ 20,650), 310 mμ (ϵ 16,050). (Found: C, 60·2; H, 4·5; OCH_3, 6·65. Calculated for $C_{22}H_{18}O_{10}$: C, 59·7; H, 4·1; OCH_3, 7·0 per cent. [A sample of isorhamnetin-3,4′,5,7-tetra-acetate obtained from G. Tappi[36] had m.p. 210–211° (block), λmax 240 (ϵ 21,750), 310 mμ (ϵ 16,700).]

Extraction of A. indica

A concentrated percolate was obtained as reported in Part III[1]. After extracting with ether, the acidic aqueous solution was basified (dilute sodium hydroxide) and again extracted with ether. This, on concentration gave yellow crystals (20 mg.) which fluoresced bright yellow in ultra-violet light and had m.p. 339–342° (decomp., block). (Found: C, 60·95; H, 4·75; N, 2·8. $C_{25}H_{23}O_{10}N$ requires: C, 60·4; H, 4·6; N, 2·8 per cent).

The aqueous alkaline layer from above was re-acidified to congo red (dilute sulphuric acid). Addition of a saturated aqueous solution of ammonium reineckate produced only a little crude reineckate (210 mg.) which gave a negligible quantity of pure material when chromatographed with dry acetone on alumina.

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